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SYNERGISTIC HYPHENATION: MERGING ANALYTICAL TECHNIQUES FOR PRECISION

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ABSTRACT

The hyphenated techniques are the combination or the coupling of the different analytical techniques such as Chromatographic (separation) techniques are combined with Spectroscopic (detection) techniques. Here the separated components of the mixture from Chromatographic technique will enter into the Spectroscopic (detection) technique through an interphase. Hence the compounds can be separated and detected simultaneously. Hyphenated techniques couple two (or more) methods such as GC-MS, LC-MS, LC-NMR, and CE-MS to solve more complex analytical problems. This technology offers faster analysis times, higher automation, better throughput, better reproducibility, and less pollution. Hyphenated techniques play a vital role in various applications in the pharmacy especially in the analysis of biomaterials, chemical fingerprinting, analytical chemistry, chemotaxonomy, carotenoids, essential oil and volatile components, alkaloids, coumarins, saponins. This article discusses the latest advances in various techniques such as GC-MS, LC-MS, LC-NMR, and CE-MS, these advanced techniques have its greatest role in enhancing the production of pure compounds.

KEY WORDS: Hyphenated technique, Analytical chemistry, LC-NMR, Dereplication, LC-IR, Reproducibility, chromatography, Spectroscopy, Automation.

INTRODUCTION

Definition:

A hyphenated technique is a combination of chromatographic (separation) and spectroscopic (detection) techniques with the help of proper interface. [1]

A couple of decades ago, the term “hyphenation” to refer to the combination of different analytical techniques was introduced by Hirschfeld in 1980.

Chromatography – produces pure or nearly pure fragments of chemical components in a mixture.

Spectroscopy – provides selective information for identification of various compounds using standards or library spectra. [2] [3]

In recent years, these hyphenated techniques have received ever-increasing attention as the primary means to solve complex analytical problems. The power of coupling separation technologies with spectroscopic techniques has been manifested over the years for both quantitative and qualitative analysis of unknown compounds in complex essential (or) natural product extracts or fractions.

The structural information obtained was leading to the identification of the compounds present in a crude sample, liquid chromatography (LC), usually a gas chromatography (GC), high-performance liquid chromatography (HPLC), or capillary electrophoresis (CE) is linked to spectroscopic detection techniques, e.g., Fourier-transform infrared (FTIR), photodiode array (PDA) fluorescence emission (or) UV–vis absorbance, mass spectroscopy (MS), and nuclear magnetic resonance spectroscopy (NMR), resulting in the introduction of various modern hyphenated techniques, e.g., CE-MS, LC-IR, LC-NMR, GC-MS, and LC-MS.

HPLC is the most widely used analytical chromatography technique for the qualitative and quantitative determination of compounds in natural product extracts. The physical connection of MS or NMR and HPLC has increased the capability of solving structural problems of complicated natural products. Because of the greater sensitivity, LC-MS has been more greatly used than LC-NMR.

These hyphenated techniques need not be between two techniques, the combining of separation and identification techniques can involve more than one separation or identification techniques, e.g., LC-PDA-MS, LCPDA-NMR-MS, LC-MS-MS, LC-NMR-MS, and the like.

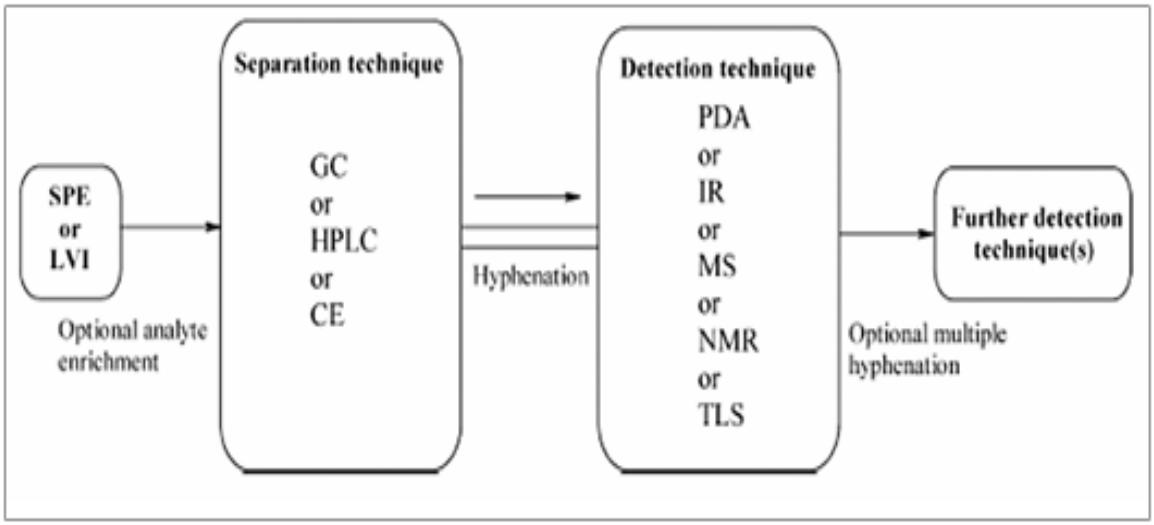

Fig1-hyphenated techniques

Nowadays, these hyphenated techniques are being accepted widely due its solution for complex analytical problems. It involves various applications and many more sophisticated methods for researchers to separate and detect many more compounds. [4] [5] [6]

ADVANTAGES OF HYPHENATED TECHNIQUES:

1. To solve complex analytical problems. Shorter analysis time.
2. Higher sample throughput, Higher degree of automation.
3. Better reproducibility.
4. Reduction of contamination because it is a closed system.
5. Increased combined selectivity and therefore higher degree of information, Accurate and fast analysis.
6. Separation of quantification at the same time. [1] [7]

DISADVANTAGES OF HYPHENATED TECHNIQUES:

1. Suitable only for volatile samples, not suitable for thermolabile substances.
2. Expensive techniques. [2]

APPLICATIONS:

1. Applicable for isolation and analysis of natural products
2. Identification of organic compounds, Used in chemical fingerprinting and quality control of herbal medicine.
3. Used for drug analysis, pesticides, herbicides detection.
4. Used in herbal drug standardisation.
5. Rapid characterisation and identification of known and new natural products. [8]

❖ **TYPES OF HYPHENATED TECHNIQUES**

It is of two types:

1. Double hyphenated techniques
2. Triple hyphenated techniques

1. Double hyphenated techniques:

- GC – MS
- LC – IR
- LC – MS
- LC – NMR
- CE – MS etc.

2.Triple hyphenated techniques:

- LC – NMR – MS
- LC – ESI – MS
- SPE – LC – MS
- LC – NMR – UV – ESI -MS
- LC - PDA – NMR – MS etc. [9]

❖ **Double hyphenated techniques**

1.GC – MS (Gas Chromatography-Mass Spectroscopy)

Introduction:

GC-MS instrument is composed of two major building blocks it includes,

1. Gas Chromatography unit and
2. Mass Spectrometer

Gas Chromatography: It is used to separate chemical mixtures into individual components (using a gas chromatograph).

Mass Spectrometry: It is used to identify the components at a molecular level (using MS detector).

With MS as the preferred detection method, and single- and ion trap and time-of-flight (TOF), triple quadrupole, mass spectrometers as the instruments most regularly used, both GC-MS and LC-MS are the most familiar hyphenated techniques in use today.

GC-MS, which is a hyphenated technique developed from the combining of GC and MS, was the first of its kind to become helpful for research and development purposes. Mass spectra obtained by this hyphenated technique provide more structural information based on the interpretation of fragmentations.

Sometimes, ionized compounds, especially those with a number of hydroxyl groups, need to be plagiarised for GC-MS analysis. The most common plagiarised technique is the conversion of the analyte to its trimethylsilyl derivative. [10] [11]

Principle:

GC-MS technology involves use of an GC, wherein the individual components in a mixture are first separated followed by separation of ions based on their mass/charge ratio in MS.

The sample solution is injected into GC inlet where it is vaporized and swept onto a chromatographic column by carrier gas (helium). The sample flows through the column and the components are separated by partition coefficient with stationary phase and carrier gas. The latter part of the column passes through a heated transfer line and ends at the entrance to ion source where compounds eluting from column are converted to their mass to charge ratio.

Components:

1.Gas Chromatography unit:

- Carrier gas (Helium)
- An injector
- An oven
- A column
- A detector

2.Mass Spectrometer:

- An ion source
- An ion guide
- A mass analyzer
- A detector [12]

Instrumentation and working:

Instrumentation:

In GC-MS, a sample is injected or introduced into the injection port of GC device, vaporized, segregated in the GC column, analysed by MS detector, and recorded. The time elapsed between injection and elution is called "retention time" (t R). The equipment used for GC-MS generally consists

1. An injection port at one end of a metal column (often packed with a sand-like material to contribute to maximum separation.
2. A detector (MS) at the other end of the column. A carrier gas (argon, nitrogen, hydrogen, helium, to name a few) propels the sample down the column.
3. The Gas Chromatography separates the components of a mixture in time. MS detector provides information that helps in the structural identification of each component.
4. The GC-MS columns can be of two types: capillary columns and packed columns and macro bore. The interface transports efficiently the effluent from the GC to MS. [13]

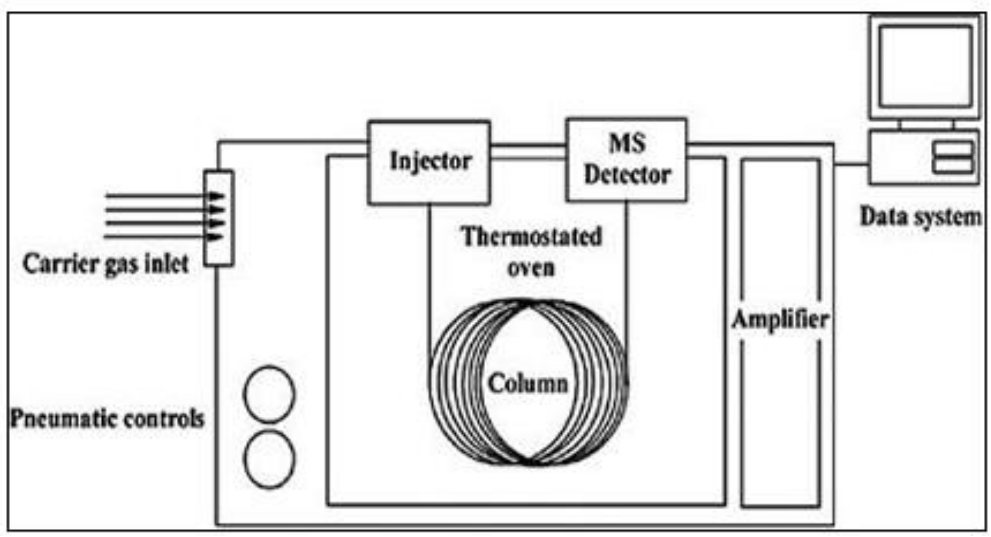

Fig: 2 gc–ms (hyphenated technique)

Working:

When the analyte vaporized by the heated carrier gas passes through the GC column, it separated from the column. Due to differences in separation or adsorption between the stationary phase and mobile phase, the elution and retention times of components differ.

The individual components of the mixture pass into the MS via the interface. This is followed by, mass spectrometry, ionization, and the determination of the mass-to charge ratio of the ions produced by each analyte using mass spectrometry. Interfaces are available in the form of effect splitters, diaphragm splitters and beam / aperture splitters, to connect the GC to the MS. The ionization process not only ionizes molecules, but also breaks them down into fragments and identifies these fragments through chemical ionization and electron collisions. The analyte molecular ions produce a spectrum of fingerprints that is different from that of other analytes. GC - MS is an essential tool in analytical chemistry. [10]

Precautions:

1. The analyte must not condense in the interface.
2. The analyte must not decompose before entering the MS ion source.
3. The gas load entering the ion source must be within the pumping capacity of the MS.

The most extensively used interfaces for a GC-MS are electron impact ionisation (EI) and chemical ionisation (CI) modes. [14]

Advantages:

1. GC having the high-resolution power than other methods.
2. This method having high sensitivity when used with thermal detectors.
3. This technique having relatively good accuracy and precision.

Disadvantages:

1. In GC only volatile samples are separated by this method.
2. During injection of the gases sample, proper attention is required. [1]

Applications:

1. LC-MS for the detection of compounds from polycyclic (non-polar) aromatic compounds to peptides and proteins.
2. LC-MS for identification and purity of compounds.
3. Used to measure pesticides, herbicides and organic pollutants for environmental monitoring.
4. This technique was used for proteomic analysis. Provides information on the molecular weight and fragmentation pattern of analyte molecules. [3]
5. Used for confirmation of purity and identity of components by measuring exact mass. [5]

2. LC – IR (Liquid Chromatography – Infrared Spectroscopy)

Introduction:

The hyphenated technique developed from the combining of an LC and the detection method infrared spectrometry (IR) or FTIR is known as LC- IR or HPLC-FTIR. While HPLC is one of the most powerful separation techniques available today, the IR or FTIR is a useful detection technique for the identification of organic compounds, because in the mid-IR region the structures of organic compounds have many absorption bands that are characteristic of particular functionalities, e.g. -COOH, -OH, and so on.

However, combination of HPLC and IR is difficult and the development in this hyphenated technique is extremely slow. In addition, as an identification technique, IR is much less sensitive compared to various other identification techniques, e.g., MS and UV. The

latest developments in HPLC-IR technology have included two basic approaches based on interfaces applied in LC-IR or HPLC-FTIR.

- One is a flow-cell approach and
- Other is a solvent-elimination approach. [15]

Principle:

LC-IR hyphenated techniques separate, identify, and quantify components in a sample using LC, while simultaneous acquiring IR spectra to provide molecular structure information.

Components:

LC:

1. Separation column
2. Pump
3. Injector
4. Detector

IR:

1. IR source
2. Interface
3. Flow cell
4. Detector

Instrumentation and working:

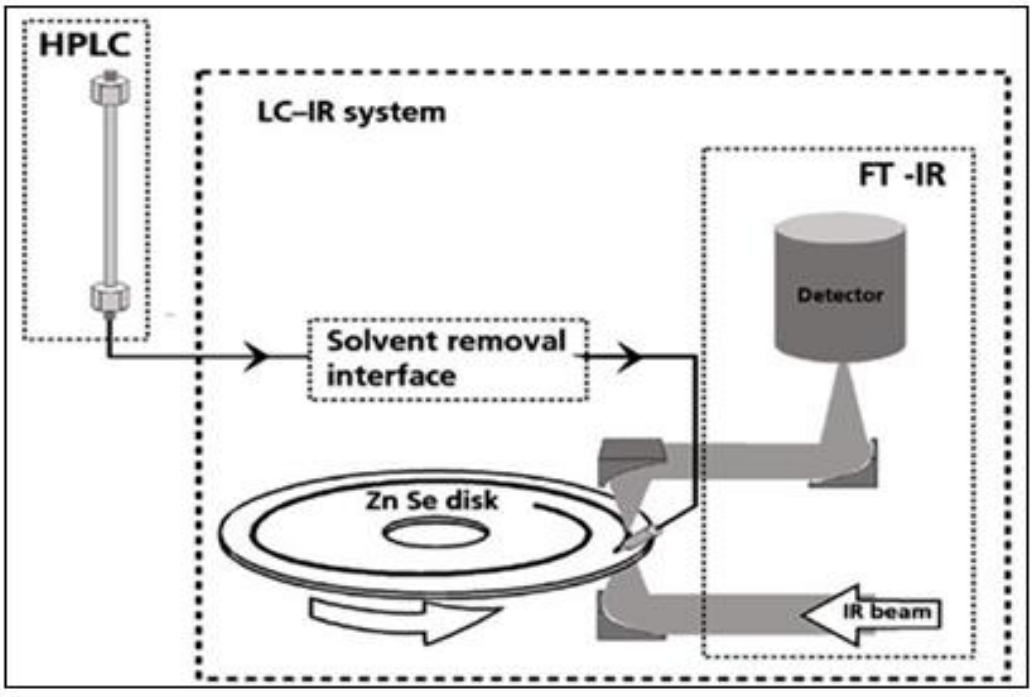

Fig-3 lc-ir (hyphenated technique)

The approach used with the flow cell in HPLC-IR is identical to that used in UV-Vis and other typical HPLC detectors. In this case, absorption of the mobile phase induces the interference of the identification of sample component absorption bands, but some transparent region of the mid-IR range produces identification possibility.

For example, if one uses a mobile phase of a deuterated solvent such as perdeuterated methanol or heavy water or, IR can monitor many organic compounds that have C-H structures in the molecules. The solvent-removal approach is the preferred choice in most of the LC-IR operations, the mobile phase solvent is eliminated, IR identification is carried out in some medium that has a transparency for IR light. Generally, KCl or KBr or salts are used for the collection of sample components in the eluent, and heating up the medium before IR compounds that have C-H structures in the molecules.

Advantages of LC-IR hyphenation:

1. Reduce analysis time.
2. LC – IR provides particular emphasis on chemometric background correction. [16]

Disadvantages of LC -IR hyphenation:

1. Selection of suitable columns and mobile phases.
2. High cost of equipment and maintenance.
3. Limited sensitivity due to IR detector limitations. [2]

Applications of LC – IR hyphenation:

1. Applications of chemometric algorithms used to improve the sensitivity.
2. Also used to improve the resolution of LC–IR signals and technical advances,
   e.g., dedicated flow cells and new light source.
3. LC-IR analysis: It is important in developing quality by design formulations for poorly water-soluble drugs. [17]

3. LC – MS (Liquid Chromatography-Mass Spectroscopy)

Introduction:

The hyphenated techniques HPLC-MS or LC-MS refers to the combining of an LC (Liquid chromatography) with a mass spectrometer (MS). [18]

Principle: The LC-MS technology involves use of an HPLC, wherein the individual components in a mixture are first separated followed by ionisation and separation of ions on the basis of their mass/charge ratio. Ionisation of separated analytes into gas phase ions.

It also involves mass analysis of ions by MS to determine molecular weight, structure and fragmentation patterns.

Components:

- A LC and a MS unit
- Interface
- Ion source
- Ion guide
- Mass analyser
- A detector

Instrumentation and working:

The LC-MS device can be associated with electro spray, particle beam, and thermal spray. Electrospray is the most commonly used interface. The syringe acts as a bridge between liquid chromatography and mass chromatography. However, independent radiators are flexible and practical. [19] [20] Changeover valves help create a functional combination of the two technologies.

Atypical automated LC-MS system consists of a double three-way catalyst with the following characteristics:

- Auto sampler
- LC system
- Mass spectrometer

The inverter usually acts as an automatic halt valve to divert unwanted portions of the eluate from the LC system into contaminants before the sample enters the MS. The ionization method used in LC-MS is a weak ionization method that usually shows only molecular ions and a small number of fragment ions. Thus, the information obtained on the structure of the compound using LC-MS method is very weak. However, this problem is currently being solved by introducing tandem mass spectrometry (MS-MS), which provides fragments with collisional dissociation of the resulting molecular ions.

The use of LC-MS MS is growing rapidly. LC-MS combines the function of MS with chemical separation LC for selective detection and confirmation of molecular identity. MS is one of the most sensitive and selective methods of molecule identification of analyte molecules. [21] [22]

Advantages:

1. High sensitivity and selectivity.
2. Structural information from fragmentation patterns.
3. Quantitation capabilities.
4. Used for identification of analyte molecules.

Disadvantages:

1. High cost of equipment and maintenance.
2. Fragmentation patterns interpretation challenges.
3. Matrix interference and contamination.
4. Limited sample throughput.

Applications:

1. LC-MS for the detection of compounds from polycyclic (non-polar) aromatic compounds to peptides and proteins.
2. LC-MS for identification and purity of compounds.
3. Used to measure pesticides, herbicides and organic pollutants for environmental monitoring.
4. Provides information on the molecular weight and fragmentation pattern of analyte molecules.
5. This technique was used for proteomic analysis. [23]

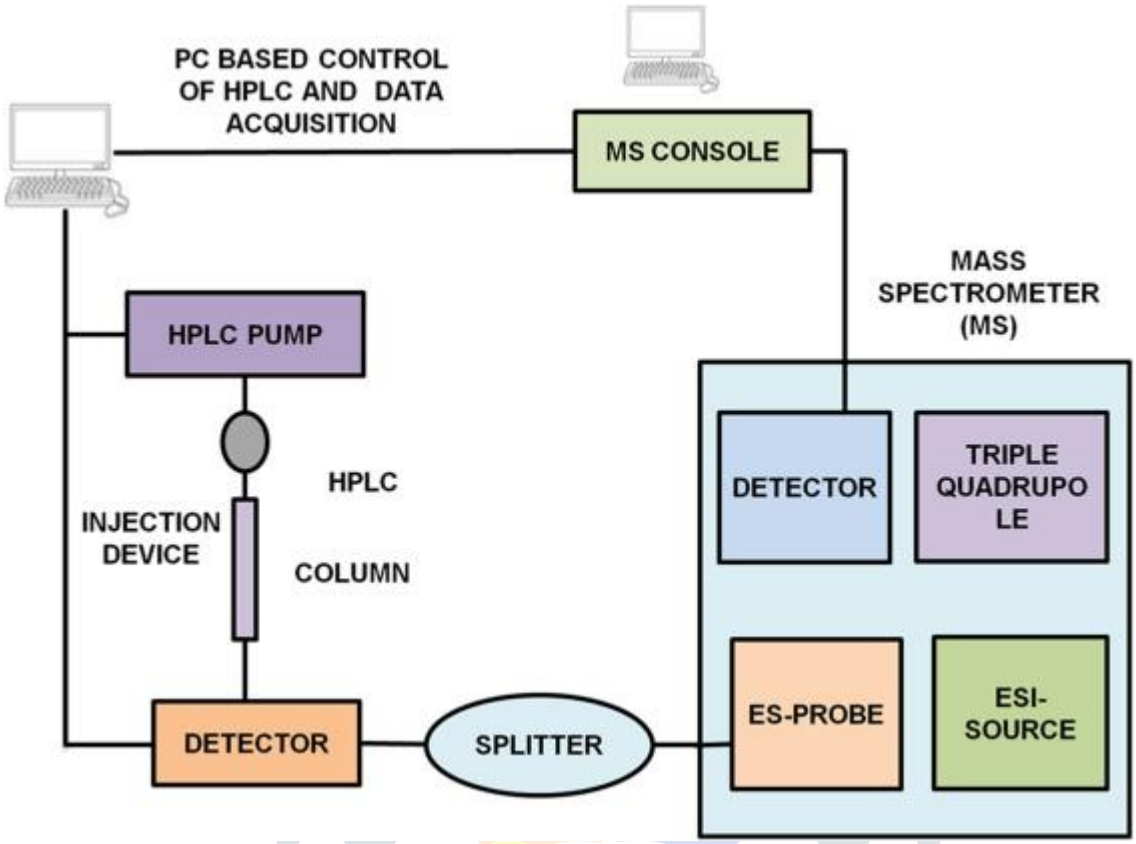

Fig-4: lc –ms (hyphenated technique)

4. LC – NMR (Liquid chromatography - Nuclear Magnetic Resonance)

Introduction:

Among the spectroscopic techniques available to date, NMR is probably the least sensitive, and yet it provides the most beneficial information regarding the structure elucidation of natural products. [24]

In this technique technological developments have allowed the direct parallel combining of HPLC systems to NMR, giving rise to the new practical technique HPLC-NMR or LC- NMR, which has been widely known for more than last 15 years. The first on-line HPLC-NMR experiment using superconducting magnets was reported in the early 1980s. However, the use of this hyphenated technique in the analytical laboratories started in the latter part of the 1990s only.

LC-NMR experiments can be performed in both stop-flow modes and continuous-flow. A wide range of bioanalytical problems can be addressed using 500, 600, and 800 MHz systems with 1H, 13C, 2H, 19F, and 31P probes. The main requirements for on-line LC-NMR, in addition to the NMR and HPLC instrumentation, are the continuous-flow probe and a valve installed before the probe for recording either continuous-flow or stopped-flow NMR spectra.

A UV-Visible detector is also used as a primary detector for Liquid-Chromatography operation. Magnetic field strengths higher than 9.4 T are recommended, i.e., 1H resonance frequency of 400 MHz for a standard LC-NMR coupling. The analytical flow cell was initially constructed for continuous-flow NMR acquisition. However, the need for full structural assignment of unknown compounds, especially novel natural products, has led to the application in the stopped-flow mode. [25] [26]

Principle:

LC separates analytes which then flow into NMR instrument. NMR detects and identifies separated analytes based on nuclear magnetic resonance.

Combined LC-NMR system provide chromatographic separation and structural identification.

Components:

- HPLC pump
- Column
- UV/Visible detector
- LC probe
- NMR console
- Software
- Interface
- Peak sampler
- NMR magnet
- Injection device
- Flow cell

Instrumentation and Working:

Generally, in LC-NMR system, the LC unit comprises:

1. Auto sampler
2. LC pump
3. Column and
4. Non-NMR detector (e.g., UV, DAD, EC, refractive index, or radioactivity).

From this detector, the flow is guided into the LC-NMR interface, which can be equipped with additional loops for the intermediate storage of selected LC peaks. The flow from the HPLC-NMR interface is then guided either to the flow-cell NMR probe-head or to the waste receptacle. Following passage through the probe-head, the flow is routed to a fraction collector for recovery and further investigation of the various fractions analysed by NMR.

In most of the LC-NMR operations, reversed-phase columns are used, employing a binary or tertiary solvent mixture with isocratic or gradient elution. The protons of the solvents of the mobile phase cause severe problems for obtaining an adequate NMR spectrum. The receiver of the NMR spectrometer is not quite able to handle the intense solvent signals and the weak substance signals at the same time. To overcome this problem, solvent signal suppression can be achieved by one of the three major methods:

- pre saturation, soft-pulse multiple irradiation or water suppression enhancement through T1 effects (WET),
- pre saturation employing a z-gradient.

This problem can also be minimized by considering the following guidelines.

1. Using eluents that have as few 1H NMR resonances as possible, e.g., H 2 O, ACN, or MeOH.
2. Using at least one deuterated solvent, e.g., D2O (approximately $290/L), ACN-d3 (approx. $1600/L), or Me OD (approx. $3000/L).
3. Using buffers that have as few 1H NMR resonances as possible, e.g., TFA or ammonium acetate.
4. Using ion pair reagents that have as few 1H NMR resonances as possible, e.g., ion pairs with t-butyl groups create an additional resonance.

Advantages:

1. Minimal sample consumption.
2. Enhanced sensitivity and selectivity.
3. Provides detailed structural information.
4. Used in identification of unknown compounds.

Disadvantages:

1. High cost of equipment and maintenance.
2. Co elution of analytes.
3. It provides limited structural information for complex molecules.
4. Flow cell design and optimisation.

Applications:

1. It provides information toward the structure elucidation of natural products.
2. The analysis of complex mixtures of all types, particularly the analysis of natural products and drug-related metabolites in biofluids.
3. This technology is used to detect pesticides, herbicides and organic pollutants for environmental monitoring. [27] [28]

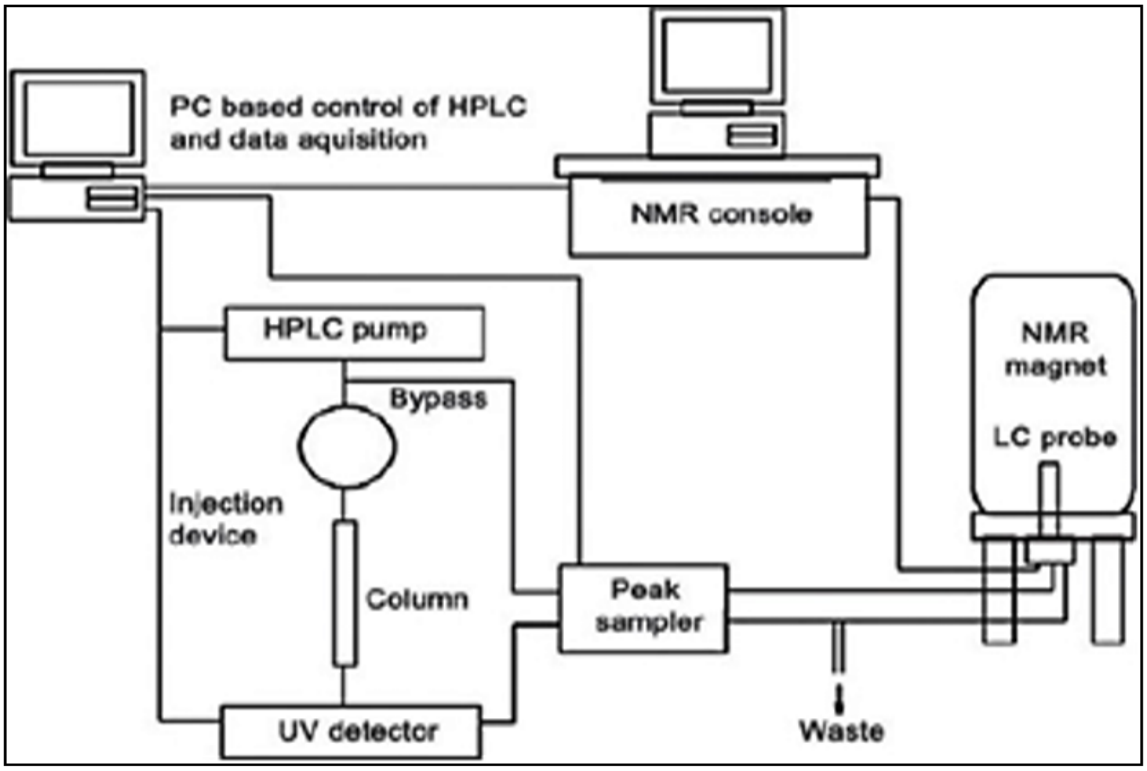

Fig-5: lc – nmr (hyphenated technique)

5.CE – MS (Capillary Electrophoresis – Mass Spectroscopy)

Introduction:

When an MS detector is linked to a CE system for acquiring on-line MS data of the separated compound, the resulting combination is termed as CE - MS. [29]CE is an automatic separation technology that was introduced in the early 1990s. CE analysis is performed under the action of an electric field in a thin tube that can quickly separate hundreds of different compounds. The flexibility and number of methods used for CE means that virtually any molecule can be isolated in this reliable way. It is commonly used to separate seeds by applying voltage to buffer-filled capillaries and to separate ions that move at different speeds depending on their size and charge when voltage is applied. A solution is considered a peak when it passes through the detector, and the area of each peak is proportional to its concentration, allowing for quantitative measurements. [30] Testing includes purity measurement, test and trace measurement. When an EM detector is connected to a CE system to obtain live EM data for isolated connections, the resulting combination is called CE-MS.

Principle:

It involves the separation of analytes by CE based on charge, size and hydrophobicity. Also include mass analysis of ions by MS to determine molecular weight, structure, and fragmentation patterns. It provides chromatographic separation based on differences in migration velocity.

Components:

- Capillary
- High voltage power supply
- Injection system
- ESI needle
- ESI interface
- Detector
- Mass spectrometer
- Electrospray Ionisation (ESI) Interface.

Instrumentation and Working:

It consists of;

1. High-Voltage Supply,
2. Capillary,
3. UV-Vis or PDA detector MS detector,
4. Buffer solution,
5. PC control. [20]

Separation is achieved through channels etched on the surface of the capillary (connected to an external high-voltage power supply) that delivers sample to ESIMS. This technique runs in full automation and offers high degree of sensitivity and selectivity. A new type of interface, known as coaxial sheath liquid CE-MS interface, has been developed recently, which allows the use of both LC-MS and CE-MS alternatively on the same mass spectrometer. [31]

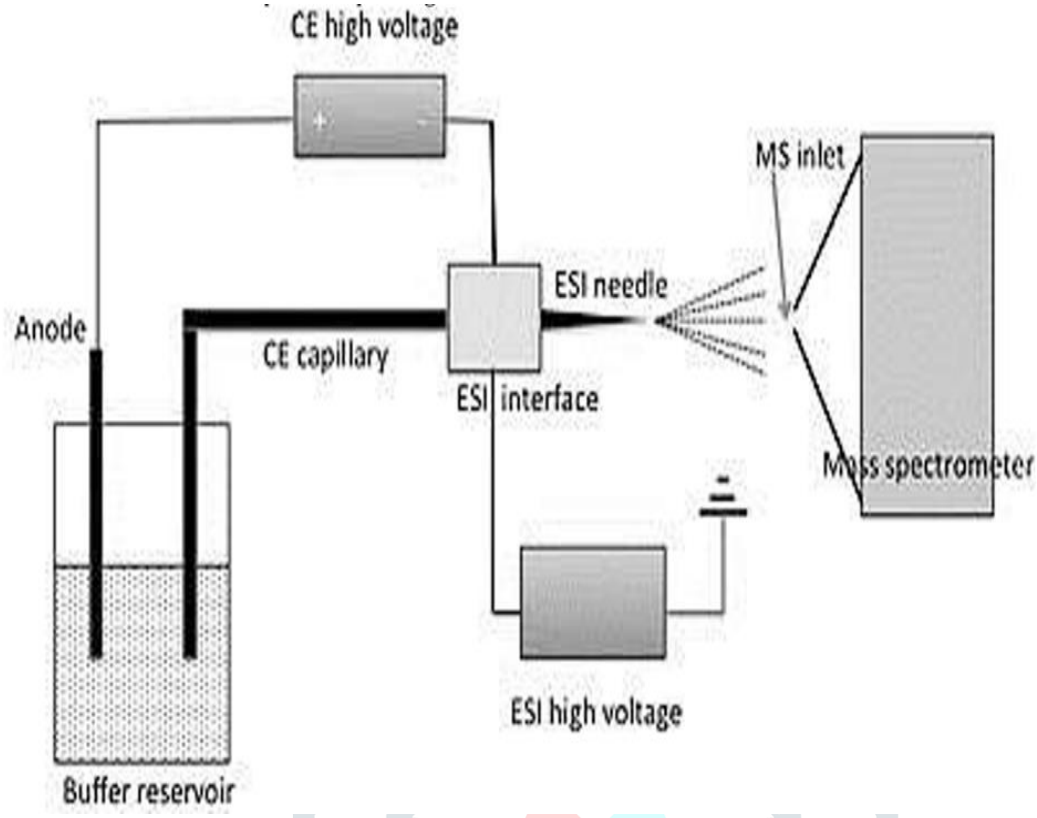

Fig-6: ce – ms (hyphenated technique)

The necessary sheath liquid is delivered by a pump that floats on the ion sprayer of the MS, avoiding any current flow toward ground. LC-MS and CE-MS modes can be switched within minutes. To obtain a stable ion spray and to avoid electrical problems, the CE power supply is used to produce the potential for the CE separation and the ESI sprayer tip simultaneously. ESIMS detection technique is generally used in most of the CE-MS systems because ESI is considered to be one of the most powerful on-line tools for the analysis of biomolecules, including natural products, providing both the molecular weight and structural characterization of analyte. [29] The optimization of the interfacing of CE with MS can be a real challenge because of the low flow rates (10–100 mL/min) required in CE, which is achieved by a make-up liquid.

Advantages:

1. High sensitivity and selectivity.
2. Fast analysis time.
3. Low sample consumption.
4. It provides structural information from MS.
5. Minimal maintenance required.

Disadvantages:

1. It provides insufficient reproducibility and selectivity.
2. It has limited compatibility with certain solvents and buffers.
3. Data processing and analysis complexity.
4. Ionisation efficiency issues.
5. High cost of equipment and maintenance.

Applications:

1. Bases and acids can be identified using non aqueous CE-MS and non-aqueous CE-MS.
2. Analyse complex arabino oligosaccharides.
3. CE-MS is a drug and biomarker discovery tool.
4. Applicable in food safety analysis. [28]

➢ APPLICATIONS OF HYPHENATED TECHNIQUES

Some examples of the application of hyphenated techniques in natural products analysis are:

I. **Isolation and Analysis of Natural Products:** Crude natural product extracts, which represent extremely complex mixtures of numerous compounds, can be analysed successfully by using appropriate hyphenated techniques. Among

the various hyphenated techniques, LC-MS are the two most extensively used for natural product analysis. LC-NMR, as well as different multiple hyphenated techniques like LC-NMR-MS have also become popular most recently.

- **Alkaloids:** GC-MS has become the method of choice for the analysis of various pyrrolizidine and quinolizidine types of alkaloids.

- **Coumarins:** The HPLC-PDA determination of coumarins, where absorption spectra are registered with a PDA detector, provides useful information about the identity of the molecule including oxidation pattern.

- **Saponins:** As saponins are highly polar compounds and difficult to volatilize, the application of GC-MS is mainly restricted to the analysis of aglycones known as sapogenins or saponins. Sometimes, precolumn derivatization of saponins can be used to attach a chromophore that facilitates UV detection at higher wavelengths. [6] [21] [22]

II. Chemical Fingerprinting and Quality Control of herbal medicine:

The use of hyphenated techniques, e.g., LC-MS, CE-MS, LC-NMR, or LC -NMR-MS, in chemical fingerprinting analysis for quality control and standardization of medicinal herbs has attracted immense interest in recent years. Generally, in the context of drug analysis, fingerprinting method is used to highlight the profiles of the sample matrix, which is often sufficient to provide indications of the source and method of preparation. In herbal medicines, the profile depends not only on the preparation processes but also on the quality of the crude herb source material. [13] A simple protocol for chemical fingerprinting of ephedra using HPLC-PDA has recently been described. Ephedra sinica (Ephedra family), also known as Ma Huang, is one of the oldest medicinal plants used in traditional herbal medicine. In Europe and the United States, E. with Sinica food supplement has become one of the top-selling weight loss and stamina products, used by over a million consumers. [32]

III. Analytical Chemistry:

It is useful in determination of drug and identification of its degraded products. It is systematically applied to monitor impurity profiles during pharmaceutical development and scaling up and supports the safety evaluation of batches used in clinical studies. [7]

IV. Chemotaxonomy:

Chemical taxonomy or chemotaxonomy is based on the principle that the presence of certain secondary metabolites is dictated by various enzymes involved in the biosynthesis of these compounds. Hence, chemical profiling of these secondary metabolites, either by complete isolation and identification, or by separation and on-line identification using modern hyphenated techniques, could provide useful information with regard to the taxonomic or even phylogenetic relationships among various species.

V. Carotenoids:

Natural substances in this group include hydrocarbons (carotene) and their oxygen-rich derivatives (xanthophylls). LC-TLS has been successfully used for the quantitative determination of carotenoids from four Phyto- and diatoxanthin, diatoxanthin and other carotenoids are also separated with higher sensitivity and selectivity than UV detection achieved by isocratic HPLC elution. This method monitors the conversion of diatoxanthin to diatoxanthin and changes in other carotenoids under various lighting conditions. LC-TLS is also a very sensitive method for measuring carotene in fish based on complement oil. [33]

VI. Essential oil and volatile components:

GC / MS has established itself as an analytical tool, especially for the analysis of nonpolar and volatile natural materials such as mono- and sesquiterpenes described how the direct expansion of GC-MS can measure approximately 130 volatiles in various herbs. They used the efficient GC- MS method using EI to isolate and assemble the de-esterified components of Chinese herbal medicine essential oils, Jilin ginseng, root and orange peel. Pestasia Atlanticaba. [34]

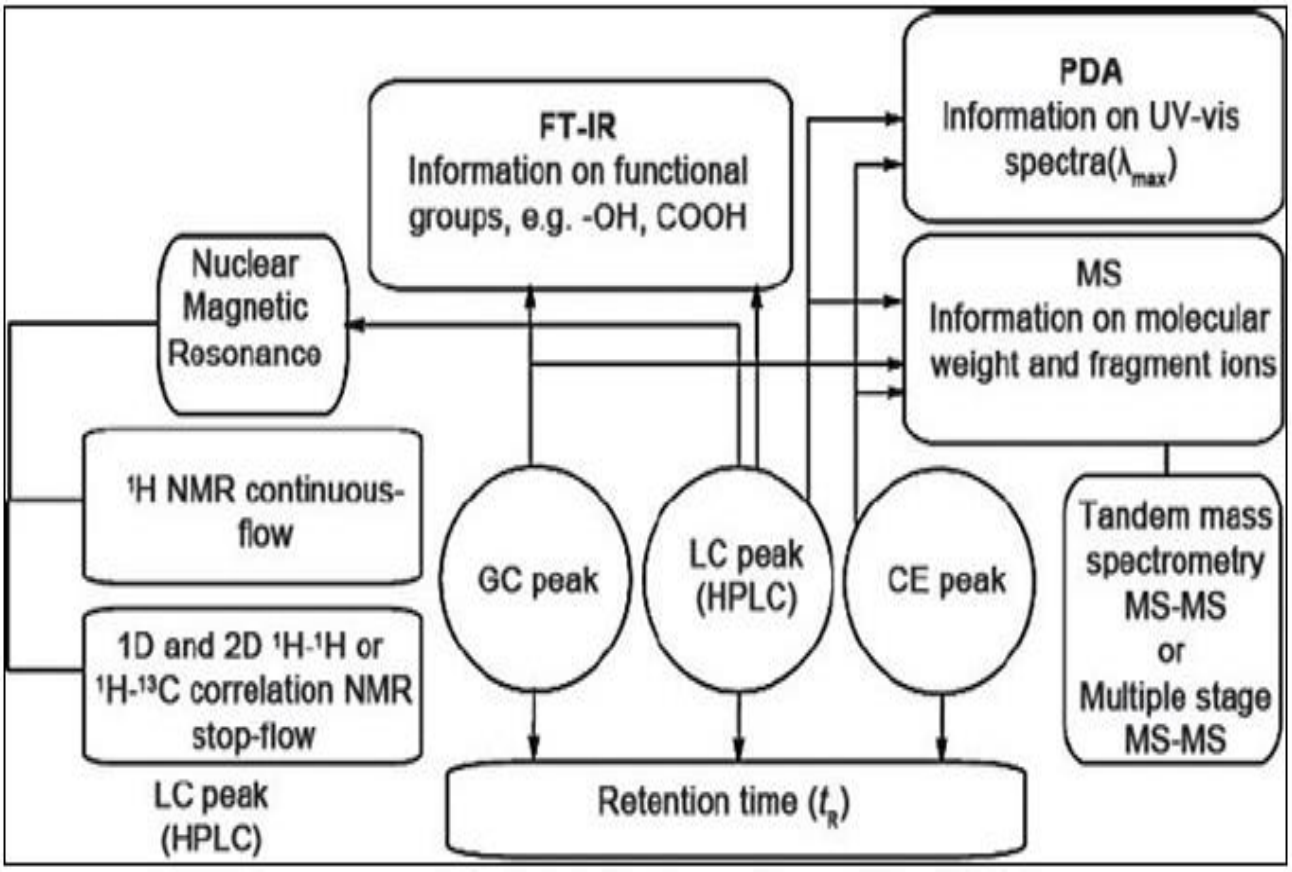

Fig-7:review of application of hyphenated technique

VII. Dereplication: Differences between previously tested or processed natural product extracts can be demonstrated by reducing the mass collection of isolated isolates requiring more accurate assessment. Isolation of natural substances based on biological tests often results in compounds known to have limited or no chemical or pharmacological significance. Technologies such as HPLC combined with UV- photodiode detection (LC-DAD-UV) and mass spectrometry (LC-MS or LC-MS-MS) provide rich on-line analytical data for pre-isolated extracts. Combined HPLC with NMR (LC-NMR) is a powerful addition to LC-UV- MS screening. [28]

CONCLUSION

The technology developed by combining separation technology and online spectroscopic detection technology is called hyphenation technology. The tremendous progress in written analytical methods. Over the past two decades has greatly expanded their application to the analysis of biomaterials, especially natural products. This article was recently developed using various methods such as GC-MS, LC-MS, LC-NMR, and CE-MS. This technology offers faster analysis times, higher automation, better throughput, better reproducibility, and less pollution.

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